pathogenesis of various age-related diseases including osteoarthritis (OA). However, the mechanisms through which high AGE diets lead to cartilage breakdown are largely unknown. ADAMTS-5 (A Disintegrin and Metalloproteinase with Thrombospondin Motifs-5, aggrecanse-2) is critical for OA progression and syndecan-4 (transmembrane heparan sulfate proteoglycan) is a key regulator of ADAMTS-5 activation. Furthermore, transcriptional regulator nuclear factor kappaB (NF-κB), activated by pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), also plays a role in the activation of ADAMTS-5. In this study we test the following hypotheses: (1) high AGE diets cause cartilage degradation, at least in part, through activation of ADAMTS-5 in chondrocytes; and (2) NF-κB is a mediator of ADAMTS-5 activation for cartilage breakdown in AGE-related osteoarthritis.

Methods: Animal studies: Following an IACUC-approved protocol, C57BL6 mice (n=12 per cohort) were fed with low or high AGE diets (low AGE diet + methylglyoxal, AGE-precursor which increases AGE formation in vivo) from birth until euthanasia at 12 or 20 months of age. Knee joints of the experimental mice were fixed in formalin and embedded in paraffin for histological evaluation of articular cartilage integrity and immunohistochemical staining. To assess whether high levels of dietary AGEs promote cartilage degradation, we performed Safranin O staining (to visualize glycosaminoglycans) and immunostaining for AGEs and ADAMTS-5 in aged mice. Immunostaining of 5-7μm thick sections was performed using polyclonal antibodies against ADAMTS-5, AGE, syndecan-4, followed by incubation with anti-rabbit secondary antibody and DAB visualization.

In vitro studies: C28/I2 human chondrocytes were treated with 0, 50, 100, 200, 400 μg/ml of AGE-BSA (Bio vision) for 8 and 24 hours. In some experiments semi-confluent cells were treated with NF-κB inhibitor (JSH23, Santa Cruz), 20μM for 24 hr or anti-syndecan-4 antibodies (Santa Cruz), 2μg/ml for 72 hr. Total RNA was isolated and qRT-PCR was performed to analyze expression of a panel of relevant genes.

Statistical Analysis. Results are presented as mean +/- SD. Statistical analysis was carried out using a Student's t-test with significance set at $P < 0.05$.

Results: Mice fed with a high AGE diet showed proteoglycan loss as indicated by reduced Safranin O staining in comparison with age-matched control mice fed with a low AGE diet. Immunostaining revealed that AGE levels in cartilage were significantly higher in the high AGE diet group compared to the low AGE group. The cartilages of high AGE diet-fed mice exhibited elevated levels of ADAMTS-5 and syndecan-4. In vitro treatment of C28/I2 human chondrocytes with AGEs mimicked the in vivo upregulation and activation of ADAMTS-5 and syndecan-4, in a time and AGE dose-dependent manner. Furthermore, inhibition of NF-κB activity resulted in suppressed levels of syndecan-4 and decreased ADAMTS-5 activity in the AGE-treated C28/I2 chondrocytes. Neutralization of syndecan-4 by anti-syndecan-4 antibodies in the AGE-treated chondrocytes suppressed activity of ADAMTS-5, but not NF-κB.

Conclusions: Our study indicates that NF-κB is a critical mediator of AGE-induced cartilage breakdown that activates ADAMTS-5 via suppression of syndecan-4. This pathway may provide a potential target for the prevention and therapeutic treatment of high AGE diet-induced OA.

220

AGGRECAN FRAGMENTS IN THE SYNOVIAL FLUID OF OSTEOARTHRITIS AND JUVENILE IDIOPATHIC ARTHRITIS PATIENTS REVEALS DIFFERENCES IN THE SPECIFICITY OF AGGRECANASE CLEAVAGE IN THE INTERGLOBULAR DOMAIN, BUT NOT IN THE CHONDROITIN-SULPHATE-RICH REGION

A. Struglics [1], S. Lohmander [1,2], K. Last [3], R. Allen [4], A.J. Fosang [3]. [1] *Dept. of Orthopaedics, Clinical Sci. Lund, Lund Univ., Lund, SWEDEN;* [2] *Res. Unit for Musculoskeletal Function and Physiotherapy, and Dept. of Orthopaedics and Traumatology, Univ. of Southern Denmark, Odense, DENMARK;* [3] *Univ. of Melbourne Dept. of Paediatrics and Murdoch Childrens Res. Inst., Royal Children's Hosp., Melbourne, AUSTRALIA;* [4] *Rheumatology Dept., Royal Children's Hosp., Melbourne, AUSTRALIA*

Purpose: Aggrecan, an extracellular proteoglycan in cartilage, is degraded by proteolysis in joint injuries and arthritis. We compared the pattern and concentration of aggrecanase-generated aggrecan fragments in the synovial fluid (SF) between patients with juvenile idiopathic arthritis (JIA), osteoarthritis (OA), young (juvenile) knee injured patients and knee healthy reference (Ref) subjects.

Methods: SF aggrecan fragments were purified by dissociative CsCl density gradient centrifugation, collecting SF-D1 fractions, from JIA (n = 12), OA (n = 4), juvenile knee injury (n = 9) and Ref (n = 11) subjects. SF-D1 pools from corresponding subject groups were also prepared. SF-D1 samples were deglycosylated and analysed by quantitative Western blot, using ADAMTS4 digested human cartilage-A1D1 fraction as ARGS-standard and bovine cartilage-A1D1 as G3-standard, with antibodies against the aggrecanase-generated ARGS neoepitope (i.e. TEGE/ARGS cleavage in the interglobular domain [IGD]) or against the aggrecan G3-domain. The SF concentration of sulfated glycosaminoglycans (sGAG) was measured by Alcian blue precipitation in JIA (n = 103 samples [from 40 patients, 0.4-21 years]), OA (n = 47, 16-89 years), juvenile knee injury (n = 7, 13-15 years) and Ref (n = 10, 19-58 years) subjects. For statistics (Mann-Whitney rank sum test) the data was expressed in pmol ARGS/ml SF, pmol chondroitin-sulphate-rich region 2 (CS2)-G3 fragments/ml SF and μg sGAG/ml SF.

Results: The SF-sGAG concentration of the JIA group was significantly lower compared with levels in the OA ($P > 0.001$), juvenile knee injury ($P = 0.003$) and Ref ($P = 0.033$) groups. By Western blot analysis, aggrecanase generated CS2-G3 fragments (i.e. GRGT-G3, GLGS-G3, AGEG-G3 and LGQR-G3 fragments of 100 - 250 kDa) were detected in the SF-D1 samples of JIA patients (Fig. 1), and a comparison between SF-D1 pools indicated only minor variations (1 - 2 fold) of the CS2-G3 fragment concentrations between JIA and the other subject groups. These results show that aggrecanases generate similar G3-containing fragments in OA and JIA patients, albeit in varying ratios. Surprisingly, very low (or no) aggrecanase generated ARGS fragments were detected in the SF-D1 samples of JIA patients (Fig. 1).The ARGS concentration of the JIA group was significantly lower compared with the OA ($P = 0.027$) and juvenile knee injury ($P < 0.001$) groups, but was not different from the Ref ($P = 0.060$) group.

Conclusions: The Western blot analysis of SF aggrecan fragments in the JIA group suggests that although the pattern of aggrecan fragments derived from the CS-rich region of aggrecan is similar in OA and JIA, there is negligible aggrecanase cleavage in the aggrecan IGD of JIA patients. This is despite the fact that aggrecan in young cartilage can be cleaved by aggrecanases in the IGD, as shown by the high level of ARGS fragments detected in the juvenile knee injury group.

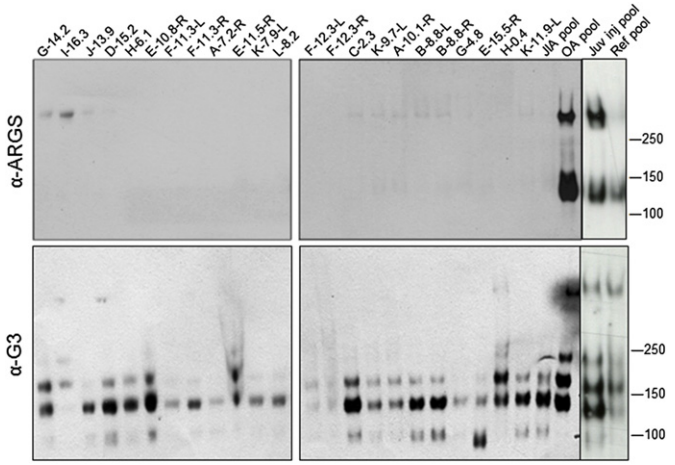

Fig 1. ARGS and G3 Western blots of JIA SF-D1 samples and SF-D1 pools (JIA, OA, juvenile knee injury and knee healthy reference). JIA patient code: A-L, different individuals; numbers, age in years; L and R, left and right knees. Loading (sGAG/lane): JIA samples, JIA- and OA-pools (1 μg, ARGS; 2 μg, G3); Juvenile injury and Ref-pools (2-3 μg ARGS and G3).

221

GLUCOSAMINE REGULATES AUTOPHAGY IN VITRO AND IN VIVO

B. Caramés, D. Brinson, M. Lotz. *The Scripps Res. Inst., La Jolla, CA, USA*

Purpose: Declining joint health as exhibited by joint pain and dysfunction may ultimately manifest as osteoarthritis (OA), the most prevalent aging-